

Studies on Enzyme Action. XVI.—The Enzymes of Emulsin.
(I) *Prunase, the Correlate of Prunasin.*

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Author's title slip :—D. M. Q.

D 8014 Emulsin (almond). Presence of prunase in

Q 1230 } Prunase and the specific nature of enzyme action
D 8012 }

D 1850 Prunasin—hydrolysis by prunase.

M 3120 Enzyme of *Prunus laurocerasus*.]

The subject has been dealt with previously in Nos. X, XII and XIII of these studies, in all of which the view was advocated that the resolution of amygdalin into two molecules of glucose and one of the cyanhydrol ($\text{CN}\cdot\text{CHPh}\cdot\text{OH}$) by almond emulsin is effected through the agency of two distinct enzymes—amygdalase and a β -glucase; the former being the enzyme by which the amygdalin is resolved into glucose and Fischer's glucoside* and the β -glucase that by which this glucoside is subsequently resolved into glucose and the cyanhydrol.

"Emulsin" occurs in the almond *fruit* and in the fruits of a variety of allied species of plants *together with amygdalin*. In continuing our studies, we have directed our attention to plant materials containing the simpler cyanophoric glucoside derivable from amygdalin, the primary object we had in view being to institute a comparison between the "enzyme" associated with the simpler glucosides and almond-emulsin, not without an expectation that in such cases we should succeed in finding the β -glucase unaccompanied by amygdalase.

The use to which we had put leaves of the cherry laurel (*Prunus laurocerasus*)† had made this plant of special interest to us; on this account, we selected it, in the first place, for examination, more particularly because Hérissé had obtained from the leaves the cyanophoric compound prulaurasin,‡ which Caldwell and Courtauld,§ in the course of these studies, have shown to be a mixture of the two stereoisomeric glucosides of the formula $\text{CN}\cdot\text{CHPh}\text{—O—C}_6\text{H}_{11}\text{O}_5$, Fischer's glucoside and sambunigrin, the latter

* Fischer, 'Ber.,' 1895, vol. 28, p. 1508.

† 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 588.

‡ 'Compt. Rend.,' 1905, vol. 141, p. 959.

§ 'Chem. Soc. Trans.,' 1907, vol. 91, p. 671.

being the glucoside characteristic of the common black-fruited elder (*Sambucus nigra*).*

Finding that it was impossible to extract an enzyme from the comminuted leaf, we resorted to the expedient of using the finely divided leaf material and found that though this was practically without action on amygdalin, it readily effected the hydrolysis of Fischer's glucoside. Our conclusion was thus verified that two distinct enzymes are concerned in the hydrolysis of amygdalin and our hope realised that it would be possible to find one or other enzyme occurring alone.

It appears to be desirable to assign a distinct name in future to the enzyme in "emulsin" by which the resolution of the simple cyanophoric glucoside is effected; as it occurs very generally in the various species of *Prunus*, we propose to term it *Prunase*; also it will be convenient to use the name *Prunasin* in speaking of the glucoside (d-mandelonitrile glucoside) which, hitherto, we have termed Fischer's glucoside.

Prunasin has been isolated from the leaf of *Prunus padus*† and of *Prunus serotina*.‡ The leaf of the laurel, *P. laurocerasus*, as already mentioned, has afforded *Prunasin* together with the isomeric sambunigrin. In the case of elder also, sambunigrin is present in the leaf. Amygdalin, apparently, on the other hand, has never been separated from leaves but only from fruit kernels; the allied complex di-glucoside vicianin, which is an arabinose-glucose derivative, also occurs in the seed, not in the leaf.§

Apparently, the enzymes are distributed in a corresponding manner, *prunase* occurring both in leaf and fruit, *amygdalase* only in minute proportion at most in the leaf but in large proportion in the seed. A typical series of results illustrating the distribution of the two enzymes in the leaf and seed are given at the head of the table in the next communication (XVII, p. 363).

We venture to think that the argument put forward in previous communications is now fully justified and that it must be regarded as proved that "almond-emulsin" is a mixture of enzymes two of which are successively concerned in the resolution of amygdalin. Failure to resolve amygdalin must be considered therefore to be primarily a proof that *amygdalase* is not present but in no way as an indication that the enzyme is absent which can only come into operation after *amygdalase* has acted. Evidence of the presence of this latter enzyme is afforded by the resolution of *prunasin*. -

* Bourquelot and Danjou, 'Compt. Rend.,' 1905, vol. 141, pp. 59 and 598.

† Hérissé, 'Arch. Pharm.,' 1907, vol. 245, p. 641.

‡ Power and Moore, 'Chem. Soc. Trans.,' 1909, vol. 95, p. 243.

§ Bertrand, 'Compt. Rend.,' 1906, vol. 143, pp. 832 and 970.

The introduction of prunasin, in place of amygdalin, as a test for prunase therefore constitutes an important advance in technique.* If amygdalin be used as a test material, assuming that it is hydrolysed only by the two enzymes amygdalase and prunase, the determination of the amount of hydrogen cyanide that is liberated serves as a means of estimating the combined activity of these two enzymes; to ascertain if the former be present without the latter, it is necessary to ascertain whether glucose be set free.

But there is another possibility to be considered, since it has been shown by Giaja† that amygdalin may be resolved into a diglucose, hydrogen cyanide and benzaldehyde by means of the gastric juice of the snail. It is to be supposed that this enzyme occurs in plants, as Bertrand succeeded in converting vicianin into hydrogen cyanide, benzaldehyde and the arabinoglucose, vicianose, by means of an enzyme present in the seeds of *Vicia angustifolia*.

In view of the results now brought forward, it may be desirable to point out again‡ that the results obtained on using emulsin as a synthetic agent cannot be regarded as due to a single enzyme. We are engaged in making synthetic experiments with the simpler materials now at our disposal.

The high values given under prunasin in Table I (p. 364)—which are discussed in the next communication—are indicative inferentially of the presence of prunase, on the assumption that (in the absence of linase) prunase alone is able to hydrolyse prunasin. Until it has been shown that such is the case, they must be subject to this reservation.

Our desire is, we may say, to confine the term *Prunase* eventually to the specific enzyme which is the true correlate of *Prunasin*. It may be well to add that we apply the term β -glucase to enzymes generally that are able to resolve β -glucosides and are controlled by glucose—in other words, to enzymes which are adapted to the glucose section of the β -glucosides.

As the conclusions which we have formulated in earlier numbers of these studies are to some extent called in question by Bayliss in Note E, in the second edition of his monograph 'On the Nature of Enzyme Action,' we may refer briefly to his criticisms. The results now brought forward should remove some of his difficulties. Bayliss is inclined to question the doctrine that enzymes are strictly specific agents. In our opinion—and we have lived with them during so many years that we claim to have some feeling on the subject—the evidence is overwhelming that they are, especially as there

* Compare 'Proc. Physiol. Soc.,' 1910, xxxiii; 'J. Physiol.,' vol. 40.

† 'Compt. Rend.,' 1910, vol. 150, p. 793.

‡ Compare No. XII, p. 323.

is reason to believe that we always deal with a variety of enzymes when using natural products.

Bayliss appears to have been impressed by the contentions advanced in a lengthy degree dissertation published in the '*Zeitschrift für physikalische Chemie*'* by Fajans, who bases his contentions on experiments which apparently have no connexion with the action of enzymes.

Bredig and Fajans have found that, on heating the two isomeric optically active *d* and *l* camphorcarboxylic acids with an optically active base, the one acid undergoes decomposition into camphor and carbon dioxide more readily than the other. It is assumed by Fajans that the optically active base functions as a catalyst—just as an enzyme does—and that whilst both acids are decomposed, the base acts preferentially, so that one acid is decomposed by it to a greater extent than the other acid. The assumption that in such a case the action is catalytic and comparable with that exercised by an enzyme is purely gratuitous, however.

Fajans himself admits that the salt is the substance that undergoes decomposition. As the salts formed from two corresponding optically active acids and an active base would not be optical opposites, the base, presumably, being differently situated in the two salts, would exercise a different attractive force in loosening carbon dioxide; it is not surprising, therefore, that the two salts undergo decomposition at different rates.

Fajans asserts that every order of difference is to be found in the behaviour of an enzyme towards optical isomers but this is definitely not true. A number of the cases to which he refers are not to the point—thus it is in no way established that the oxydases are enzymes and the fact that moulds are able to utilise both components of racemic amino-acids is easily accounted for in view of the ease with which these acids are racemised by ordinary agents. In the case of lipase, there is no reason to correlate the activity of the "enzyme" with optical activity—it is a selective agent only in the sense that it is a specific hydrolyst of ethereal salts of carboxylic acids. In point of fact, the only definition that can be given, at present, of an enzyme that will separate enzymes from other hydrolytic agents is one which involves the assumption that they act selectively.

* 1910, vol. 73, pp. 25—96.